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MINIMAL INHIBITORY CONCENTRATIONS OF QUINOLONES GROUP TO SOME AVIAN MYCOPLASMAS

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SUMMARY

Quinolones group (Ciprofloxacin, Enrofloxacin and Norfloxacin) was found to have antimicrobial activity (using growth inhibition test) on Mycoplasma synoviae, M. pullorum and M. iowae, while M. gallisepticum strains (S6, R, PG31 and F strains), M. gallinaceum, M. galinarum and M. iners were less sensitive. All the examined strains were sensitive to Lincospectin. Streptomycin had less effect on M. synoviae, M. pullorum. M. gallinarum and M. iners, while M. gallisepticum strains, M. gallinaceum and M. iowae were resistant.

Minimum inhibitory concentrations (MICs) of Ciprofloxacin and Enrofloxacin were between 0.006 Ug/ml (sensitive) for all the tested strains. In the case of Norfloxacin, most of the examined strains were sensitive (0.024-0.048 Ug/ml) except M. gallisepticum (S6, R and F strains) and M. iowae were less sensitive (0,097-0.39 Ug/ml).

Streptomycin had less effect on most tested strains (0.39-1.56 Ug/ml), while M. iowae was resistant.

INTRODUCTION

Leclerc (1971) recorded that Tylosin has the strongest inhibitory effect on pathogenic avian mycoplasmas. Senterfit (1983) tried to determine the minimum inhibitory concentration (MIC) for evaluation of the efficacy of antimicrobial drugs using metabolic inhibition test (MIT). El-Ebeedy et al. (1987) tested the sensitivity of some mycoplasma and ureaplasma isolates to certain antibiotics using disc growth inhibition (GIT) and metabolic inhibition (MIT) tests. Mycoplasma gallisepticum and M. gallinarum were sensitive to Spectinomycin and Apramycin. El-Shater (1987) found that M. gallisepticum, M. gallinarum and M. iners were sensitive to Alplucin, Lincomycin, Spectinomycin and less sensitive to chloramphenicol and Apramycin.

Tilmicosin, a new semi - synthetic macrolide antibiotic, ha in vitro activity against M. galisepticum with a minimum inhibitory concentration of 0.048 Ug/ml (Shryock et al., 1994). Shaker (1995) found that the most effective antibiotics against M. gallisepticum and M. synoviae were Lincospectin and Kitomox.

This study was conducted to determine the minimum inhibitory concentrations (M. I. Cs) of

Quinolones group to avian mycoplasmas by growth inhibition (GI) and metabolic inhibition (MI) tests.

MATERIAL AND METHODS

Ten reference avian mycoplasma strains, obtained from Dr. El-Ebeedy, Directory of Animal Health Research Institute, Dokki, Egypt, were used in the present work. Media used for the propagation of mycoplasma were prepared as described by Erno and Stipkovits (1973). Mycoplasma synoviae was propagated in Frey's broth medium (Frey et al., 1968).

Quinolones group, obtained from Sigma, Ltd., England (Ciprofloxacin, Enrofloxacin and Norfloxacin) were prepared as stock solutions to contain 1000 Ug/ml of drug in sterile distilled water, a small quantity of IN NaOH was used as described by Anderson (1970). Discs were prepared using Whatman antibiotic assay discs (6mm) 25 Ul of stock solution for each drug of Quinolones group, dispensed for each disc, left to dry then used directly or kept till use.

Lincospectin and Streptomycin sensitivity discs produced by Upjhon Company, USA and Oxoid Laboratories, England, respectively, were also used.

The growth inhibition (GI) test was carried out according to Clyde (1964). The mycoplasma strains were tested on PPLO agar media using the running drop technique and disc of antibiotics with several dilutions of mycoplasma broth culture. The plates were incubated at 37°C for 2-5 days and examined both macroscopically and microscopically for the inhibition of growth of colonies. Interpretation of the results was according to El-Ebeedy et al. (1987).

Metabolic inhibition (MI)test was done as described by Senterfit (1983). The test was performed in duplicate, the antimicrobials were tested in serial twofold dilutions. The end point (MIC) was read as the least concentration of a drug that completely inhibited growth (no colour change of phenol red). This typically occurs after 1 to 2 days and results are expressed Ug/ml (Bradbury et al., 1994).

RESULTS

Results of in vitro assay of sensitivity of avian mycoplasmas to Quniolones group are presented in Table (1). Mycoplasma synoviae. M. pullorum and M. iowae were found to be sensitive for the three drugs of Quniolones group (Ciprofloxacin, Enrofloxacin and Norfloxacin), while M. gallisepticum strains, M. gallinaceum, M. gallinarum and M. iners were less sensitive.

All tested strains were sensitive to Lincospectin. Mycoplasma synoviae, M. pullorum, M. gallinarum and M. iners were less sensitive to Streptomycin, while M. gallisepticum strains, M. gallinaceum and M, iowae were resistant.

Table (2) shows the M. I. Cs of Quniolones group to avian mycoplasmas. In the case of Ciprofloxacin and Enrofloxacin, all tested strains were sensitive (0.006 - 0.048 Ug/ml). Mycoplasma gallisepticum (PG31 strain)

Table (1): Antibiotic sensitivity test of avian mycoplasmas using growth inhibition test

Mycoplasma species	Ciprofloxacin (25 Ug)	Enrofloxacin (25 Ug)	Norfloxacin (25 Ug)	Lincospectin (20 Ug)	Streptomycin (10 Ug)
M. gallisepticum					
S6 strain	*12	11	8	10	4
R strain	12	11	9	11	4
PG31 strain	12	12	9	11	4
F strain	9	9	8	12	4
M. synoviae (WVU)	18	20	18	14	8
M. pullorum (CKK)	16	19	12	13	7
M. gallinaceum (DD)	11	10	8	10	4
M. iowae (I)	19	19	16	14	2
M. gallinarum (PG18)	9	12	10	13	8
M. iners (PG30)	12	10	8	10	7

*Inhibition zone (millimeter)

	Quinolones	Lincospectine	Streptomycin
Sensitive	≥ 12	≥ 10	≥ 10
Intermediate	8 - 11	7 - 9	7 -9
Resistant	≤ 7	≤ 6	≤ 6

Interpretation of results was done according to EL - Ebeedy et al. (1987) .

Msynoviae, M. pullorum, M. gallinaceum, M. gallinarum, M. iners were sensitive to Norfloxacin (0.024-0.048 Ug/ml), while Mycoplasma gallisepticum (s6 strain) was less sensitive (0.39 Ug/ml).

Most tested strains were found to be less sensitive to Streptomycin, exceptM. iowae was resistant.

Table (2): Minimal Inhibitory Concentration (MICs) of Quinolones group to avian mycoplasmas

Antibiotic	Mycoplasma gallisepticum				M-synoviae	M. pullorum	M. gallinaceum	M. iowae	M. gallinarum	M. iners
	S6 strain	R strain	PG31 strain	F strain	(WVU)	(CKK)	(DD)	(I)	(PG18)	(PG30)
	**CFU 10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8
Ciprofloxacin	*0.048	0.024	0.024	0.048	0.024	0.006	0.012	0.048	0.012	0.024
Enrofloxacin	0.024	0.012	0.012	0.048	0.024	0.006	0.012	0.048	0.012	0.024
Norfloxacin	0.39	0.097	0.024	0.097	0.024	0.048	0.024	0.097	0.024	0.048
Streptomycin	0.39	0.39	0.78	1.56	1.56	1.56	0.78	0.0	1.56	1.56

*MIC (Ug/ml)
**CFU=Colony Forming Unit
Sensitive: 0.006 - 0.05 Ug/ml
Intermediate: 0.4-2.0 Ug/ml
Resistant: > 3.0 Ug/ml
Interpreation of results was according to Bradbury et al. (1994).

DISCUSSION

Among ten reference mycoplasma strains tested in this study, Mycoplasma synoviae, M. pullorum and M. iowae were found to be sensitive to Ciprofloxacin, Enrofloxacin and Norfoxacin, followed by M. gallisepticum strains. M. pullorum, M. gallinarum and M. iners.

Shaker (1995) recorded that M. synoviae and M. gallisepticum field and reference strains were sensitive to Lincospectin and Kitamox. Lincospectin was effective for all tested strains.

The determination of MIC has been reference point in evaluating the efficacy of antimicrobial drugs by in vitro tests (Hariharan and Barnum, 1974). In the present study, Ciprofloxacin, Enrofloxacin had the highest effect (MICs 0.006-0.048 Ug/ml) for all examined strains. In the case of Norfloxacin, a good result was obtained with the most tested strains (MICs 0.024 - 0.097 - Ug/ml), while M. gallisepticum (s6 strain) was less sensitive (MIC 0.39 Ug/ml).

Streptomycin had the least avitivity (MIC 0.39 - 1.56 Ug/ml) for the most examined strains, while M. Iowae was resistant. El-Ebeedy et al. (1987) found that M. gallisepticum and M. gallinarum were less sensitive to Streptomycin.

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